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NOTES

Effect of Amphotericin B Methyl Ester on Vesicular Stomatitis Virus Morphology

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The water-soluble methyl ester of amphotericin B inactivates vesicular stomatitis virus in association with morphological alterations of the envelope.

The biological effects of the polyene-macrolide antimicrobial agents depend upon a high affinity for sterols in the cell membrane of susceptible organisms (6). This class of compounds is active against fungi, mycoplasmas, and protozoa, but not bacteria, because the bacterial cell membrane does not depend upon sterols for stabilization. Recently, a water-soluble derivative of amphotericin B, the methyl ester (AME), has been shown to possess antiviral activity against certain enveloped viruses, including the rhabdovirus vesicular stomatitis virus (VSV; 3, 5, 7). Antiviral activity could not be demonstrated against the unenveloped echovirus type 11 or adenovirus type 4 (3). We report here on the morphological changes that result in VSV after exposure to AME.

The Indiana strain of VSV was kindly supplied by R. S. Chang, School of Medicine, University of California, Davis. Virus stocks were grown in primary chicken embryo cells. Crude virus was clarified by centrifuging at 121 × *g* for 15 min. Virus was sedimented from the supernatant solution by centrifuging at 21,000 × *g* for 90 min in a Spinco 30.1 rotor. The virus pellet was suspended in 1 ml of TEN buffer [Tris (hydroxymethyl) amino - methane - hydrochloride (0.01 M)-ethylenediaminetetraacetic acid (0.001 M)-NaOH (0.1 M) pH adjusted to 7.3 with HCl (1 M)] and layered onto a preformed continuous 10 to 50% (wt/vol) sucrose gradient. The sucrose gradient was centrifuged in a Spinco SW41 rotor at 112,500 × *g* for 120 min, and the fraction containing the light-scattering B band (infectious virus) was collected. The titer of the concentrated VSV as determined by the plaque method in L-cells was 5.0×10^9 plaque-forming units per ml. AME, as the hydrochloride, was kindly supplied by C. P. Schaffner, the Waksman Institute of Microbiology, Rutgers University, New Brunswick, N. J. Mixtures composed of one-fourth part of concentrated VSV and three-fourths part of AME at a final concentration of 100 μg/ml were prepared and incubated for 60 min at 4 and 37°C, respectively. Hanks balanced salt solution was used in the controls. The mixtures were agitated with a Vortex mixer halfway through the incubation period. After incubation, samples of the mixtures were removed for virus assay and electron microscopic examination. A drop of the mixture was placed onto a carbon-coated Formvar grid for 10 min. The excess fluid was blotted off with a filter paper, and the sample was stained with neutral 2.5% phosphotungstate for 2 min before it was examined in an AEI EM-6B electron microscope.

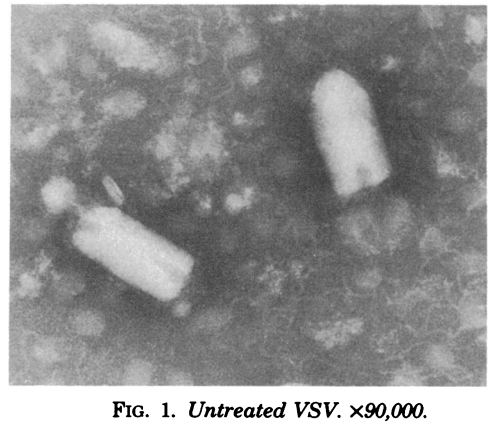

FIG. 1. *Untreated VSV.* ×*90,000.*

Exposure of VSV to AME at a concentration of 100 μg/ml resulted in a 100- to 1,000-fold decrease in infectivity, depending upon the temperature at which the drug-virus interaction took place (Table 1). In comparison with the

TABLE 1. *Effect of AME on VSV morphology and infectivity*

Temp	Sample	Titer (PFU[a]/ml)	Fields examined	Virions counted	No. Deformed	% Deformed[b]
4°C	AME	3.4×10^7	18	136	71	52.2
	Control	2.0×10^9	10	156	1	0.6
37°C	AME	1.0×10^4	24	211	178	84.3
	Control	1.4×10^7	10	129	5	3.8

[a] PFU, Plaque-forming units.

[b] In chi-square analysis for preparations exposed to AME at both temperatures, $P < 0.005$.

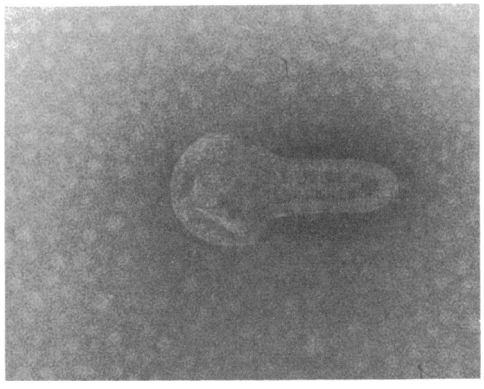

FIG. 2. *VSV exposed to AME (100 μg/ml) for 60 min at 37°C. ×100,000.*

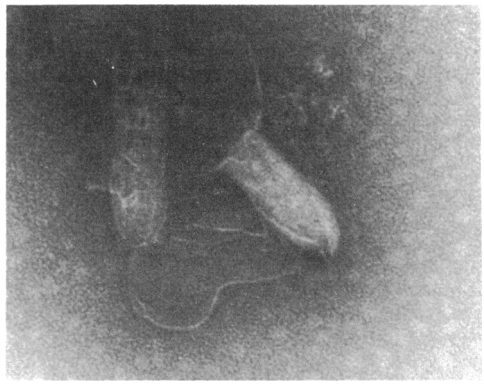

FIG. 3. *VSV exposed to AME (100 μg/ml) for 60 min at 4°C. ×100,000.*

typical bullet-shaped appearance of the control preparations (Fig. 1), many damaged particles were seen after exposure to AME. The most characteristic change was a bulbous deformity seen at the blunt end of the virion (Fig. 2 and 3). A similar appearance has been described for two plant rhabdoviruses, potato yellow dwarf virus and rice transitory yellowing virus (2, 4). It has been speculated that the spherical deformity in these plant viruses is an artifact of fixation. In the case of VSV, deformed particles were seen significantly more often after treatment with AME than in control preparations, both at 4 and 37°C (Table 1). A statistically significant ($P < 0.005$) increase in deformed particles was seen in preparations exposed to AME at 37°C in comparison with AME exposure at 4°C. It is unlikely that the morphological alterations of the virion result from the conditions of preparation, because control samples prepared in the same manner as drug-treated viruses appeared to be undamaged. Studies by Bates and Rothblat (1) demonstrated that changing the sterol composition of the VSV envelope from cholesterol to desmosterol did not alter its stability or infectivity. Whereas the kind of sterol present does not appear to affect the infectivity of VSV, the polyene antibiotics have a high affinity for all sterols. Our data are consistent with the interpretation that the water-soluble AME interacts with sterols of the viral envelope, resulting in morphological alterations of the virion and loss of infectivity.

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LITERATURE CITED

1. **Bates, S. R., and G. H. Rothblat.** 1972. Incorporation of L-cell sterols into vesicular stomatitis virus. J. Virol. **9**:883-890.
2. **Chen, M., and E. Shikata.** 1971. Morphology and intracellular localization of rice transitory yellowing virus. Virology **46**:786-796.
3. **Jordan, G. W., and E. C. Seet.** 1977. Antiviral effects of amphotericin B methyl ester. Antimicrob. Agents Chemother. **13**:199-204.
4. **MacLeod, R.** 1968. An interpretation of the observed polymorphism of potato yellow dwarf virus. Virology **34**:771-777.
5. **Mechlinski, W., C. P. Schaffner, and D. P. Bonner.** 1972. Communications to the editor. J. Antibiot. **25**:256-262.
6. **Norman, A. W., A. M. Spielvogel, and R. G. Wong.** 1976. Polyene antibiotic-sterol interaction. Adv. Lipid Res. **14**:127-170.
7. **Stevens, N. M., C. G. Engle, P. B. Fisher, W. Mechlinski, and C. P. Schaffner.** 1975. *In vitro* antiherpetic activity of water-soluble amphotericin B methyl ester. Arch. Virol. **48**:391-394.